1.1 Scientific Abstract

Prostate cancer is the second leading cause of cancer death in men in the United States (Wingo et al 1996). The incidence of this cancer has increased dramatically over the past 25 years, and this has been attributed in part to improved detection of subclinical carcinomas during transurethral resections of the prostate (TURP) (Potosky et al 1990), but also to improvements in screening for elevated prostate-specific antigen (PSA) (Oesterling et al 1991). PSA, a prostate-derived serine protease, is found in high concentrations in the seminal fluid and can also be detected in the serum. PSA levels have been shown to increase with increasing age and have also been correlated with the development of benign prostatic hypertrophy (BPH) and with prostate cancer (Wingo et al 1996; Hanks et al, 1993a; Aumuller et al 1990).

Treatment for early stage (T_1, T_2) organ confined prostate cancer has generally consisted of either radical prostatectomy or radiation therapy (external beam or implant), and has been relatively effective, resulting in good, long-term disease-free intervals and overall survival (Par-tin et al 1993; Trapasso et al 1994; Catalona et al 1994; Hanks et al, 1993b). The treatment of patients with locally advanced prostate cancer (T_3, T_4) has been less effective (Hanks et al 1993b; Blasko et al 1994). Patients receiving radiation therapy with or without adjuvant hormonal ablation therapy have a 60% probability of recurrence locally, and many will eventually develop metastatic disease (Pilepich et al 1995a; Pilepich et al 1995b).

Local recurrence (T_3, T_4) of prostate cancer after definitive radiotherapy has been estimated at an incidence of slightly over 50,000 new cases per year in the US (Moul et al 1998). The treatment options for these patients are generally not curative and include observation, hormonal ablation, salvage prostatectomy, cryoablation, thermal rod therapy, repeat external beam irradiation or localized brachytherapy (radiation seed implantation), and investigational therapies (such as gene therapy, etc.) (Letran et al, 1998). In addition, there are very few controlled clinical studies in this group of patients assessing the various treatment modalities. The majority of these surgical or radiation therapies can cause significant patient morbidity (e.g. complete impotence, incontinence, fistulae, etc.), and none have been proven to affect overall survival. Thus, local therapies that eradicate disease without incurring significant morbidity are needed.

If, after use of these modalities, the disease metastasizes and continues to progress, current first-line chemotherapy is not generally curative, and therapy is usually aimed at palliation and pain control using a variety of agents including estramustine, vinblastine, etoposide, mitoxantrone, and glucorticoids alone or in combination. However, if patients fail these regimens, at present, there is no further therapy which can be offered. New therapeutic modalities are therefore actively being sought for these patients.

Human adenoviruses were first cultured from the tonsils and adenoids of children in 1953 (Rowe et al 1953). Adenovirus type 5 (Ad5) is associated with a self-limiting cold or flu-like syndrome in humans. In immunosuppressed individuals it has also been associated with renal impairment and hepatic necrosis, and with gastric erosions (Zahradnik et al 1980). Ad5 has been reported to have little or no oncogenic potential in mammals (Horwitz et al 1990). A recent serologic survey has revealed that 57% of the adult population in the U.S. has neutralizing antibodies to Ad5 (Schulick et al 1997).

Several attempts have previously been made to exploit the cell killing properties of replication-competent adenoviruses in the treatment of cancer (Smith et al 1956; Ganly et al 1997; Moore et al 1954; Kim et al 1996; Clayman et al 1997). Smith et al administered local injections of Ad5 and other serotypes of adenovirus to patients with cervical cancer. Tumor necrosis and cavity formation were observed (Smith et al 1956). Adverse events included fever, photophobia, and malaise lasting seven to nine days. More recently, other studies have been conducted, including a study of the local injection of ONYX-015™, a genetically modified replication-competent adenovirus, which was performed in patients with refractory cancer of the head and neck and reported at the American Society of Clinical Oncology (ASCO) (Ganly et al 1997). This virus is an E1B-deleted group C adenovirus that selectively replicates in and lyses p53-deficient tumor cells. When the virus was combined with the chemotherapeutics cisplatin and 5-fluorouracil, nine out of ten patients experienced a greater than 50% reduction in tumor size, with two complete remissions. Adverse events were reported to be mild to moderate in severity.

Calydon Inc. has constructed several attenuated, replication-competent, genetically modified Ad5 virus (ARCA™) which preferentially replicate in PSA-producing prostate cells. CN706 was the first generation virus which was constructed by removing the adenoviral E3 region of the genome (not required for replication in vitro), and inserting the recently cloned PSA promoter and enhancer elements upstream of the E1A region of the genome of the virus. The addition of the PSA promoter and enhancer elements resulted in the preferential replication of CN706 in PSA-producing prostate cancer cells.

In vivo studies in a nude mouse model have shown that intratumoral injections of CN706 can significantly retard the growth of prostate carcinoma xenografts. Treatment with CN706 (3.3×10^{11} particles on Days 0 and 3) has also been shown to result in a decline in serum PSA levels as compared to levels in control animals treated with vehicle alone. This first generation virus CN706 is currently being studied in a Phase I clinical study (Calydon Protocol CN706-001) in patients with locally recurrent prostate cancer following external beam radiotherapy (RT). Four cohorts of three patients each at dose levels of 1×10^{11} to 3×10^{12} virus particles have been treated. Thirteen patients have received treatment with CN706 and experienced only mild to moderate adverse events/toxicities. Most patients experienced some pain and/or edema at or around the injection and/or biopsy sites due to study procedures. Although there were within-patient variations in laboratory parameters, no clinically significant results were observed. All patients experienced some hematuria as a result of having an indwelling Foley catheter for two weeks. Based on the interim data analysis of Protocol CN706-001, doses of up to 1×10^{12} virus particles have been safely administered to patients with locally recurrent prostate cancer. Dose-limiting toxicities were not observed in any patient in the first four cohorts; therefore, the maximum tolerated dose (MTD) has not yet been defined.

CV787, the virus proposed to be used in this study, is a second-generation virus with much more specificity than CN706 for PSA-producing cells (Yu et al, 1999). This virus was constructed by inserting the PSA promoter and enhancer elements upstream of the E1B region of the genome of the virus and the rat prostate probasin promoter (and enhancer elements) upstream of the El A region of the genome of the virus. The insertion of these elements resulted in the preferential replication of CV787 in PSA-producing cells. In

addition, the E3 region of the genome was reintroduced into the virus in order to achieve greater cell killing.

An in vivo study comparing the efficacy of CV787 to CN706 was conducted in the athymic nude mice xenograft model. An injection of a single dose of CV787 at 1 x 10^6 particles/mm^3 or 1 x 10^8 particles/mm^3 tumor volume into the xenograft resulted in a decrease in xenograft tumor volume to 46.5% or 5.2% of baseline volume, respectively. These changes were statistically significant compared to the control animals. CV787 showed a much higher potency than CN706 in this model.

Studies of intravenous CV787 in the mouse xenograft model showed that a single dose of CV787 at 1 x 10^{11} particles per animal through tail vein injection resulted in a decrease in xenograft tumor volume to 5% of their original size at 6 weeks after treatment, and 8 of 14 mice were visually free of tumors. The serum PSA levels increased in control mice, whereas the PSA levels decreased to less than 5% of their starting value within 3 weeks in mice injected with CV787.

A study was conducted to compare the acute toxicity and inflammatory potential of CV787 and CN706 in immunocompetent mice (C57 BL/6) with subcutaneous (SC), intradermal (ID), and intravenous (IV) administration. The toxicity of CV787 in this study was no more severe than that of CN706. SC and ID administration of both viruses at a single relatively large dose of 1 X 10^{11} virus particles per animal produced minor inflammation, localized to the injection sites. No difference in severity was observed between the two treatments. Acute liver toxicity following IV administration of 1 x 10^{11} virus particles per animal was confirmed by histopathology, and was less severe for CV787 than for CN706.

Biodistribution and toxicology studies have been performed with CV787 in Cotton rats (*Sigmodon hispidus*). The biodistribution study showed that the virus primarily accumulated in liver and spleen tissue, and to a lesser extent in kidney, lung, heart, and testis tissue, but was absent from brain tissue. The toxicology study showed no toxic effects other than observations of some single cell necrosis in the liver, and slightly higher spleen weights in some animals receiving CV787.

These preclinical studies have demonstrated that treatment with CV787 by direct tumor injection and by intravenous administration can result in significant reductions in tumor volume in a mouse xenograft model, and is well tolerated. CV787 represents a novel approach to the treatment of locally recurrent prostate cancer, by targeting the cytolytic effects of adenovirus to PSA-producing cells.

The proposed studies will assess the safety and tolerance and determine the efficacious dose of CV787 in patients via intraprostatic administration to patients with locally recurrent or persistent prostate cancer who have received definitive RT, and by intravenous administration to patients with metastatic hormone-refractory prostate cancer. The starting dose level will be 1x10^{12} virus particles, the same dose as that of CN706 which has been determined to be well tolerated in patients with locally recurrent prostate cancer.